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(54) Title: HEMOSTATIC COMPOSITIONS, DEVICES AND METHODS

(57) Abstract: A hemostatic composition which comprises at least one procoagulant metal ion, such as silver (I) or mercury (II), and at least one procoagulant biopolymer, such as collagen, thrombin, prothrombin, fibrin, fibrinogen, heparinase, Factor VIIa, Factor VIII, Factor IXa, Factor Xa, Factor XII, von Willebrand Factor, a selectin, a procoagulant venom, a plasminogen activator inhibitor, glycoprotein IIb-IIIa, a protease, or plasma. The composition in the form of a paste, dough, glue, liquid, lyophilized powder or foam, may be provided, for application to a wound. A hemostatic device is also described which comprises a hemostatic composition as described above. The device may be in the form of, for example, a plug, bandage, gauze, cloth, tampon, membrane or sponge. Methods are also provided for prophylaxis or treatment of bleeding at a site by application to the site of the composition or device as described.

HEMOSTATIC COMPOSITIONS, DEVICES AND METHODS

BACKGROUND OF THE INVENTION

Uncontrolled hemorrhage from any type of wound, whether accidental or iatrogenic, may result in dire consequences. In particular, bleeding attendant with arterial damage is particularly troublesome, especially in an individual with a coagulopathy, congenital, the result of disease, or induced by administration of anticoagulants. Moreover, recovery from surgical and other procedures involving transarterial cannulation, particularly in anticoagulated individuals, presents a challenge to the medical profession as such individuals often present prolonged bleeding times from such wounds, necessitating close monitoring and increased length of hospital or in-patient stays. In such patients, manual pressure, pressure bandages, sandbags, and other means have been used to hasten hemostasis at the wound site. Not only is the excessive bleeding potentially dangerous to the patient, but the burden of the excess attention to the wound is dangerous to the already overburdened health care system.

The foregoing discussion is also pertinent to arterial and other major hemorrhagic wounds suffered from traumatic injury such as those inflicted on the battlefield, as a result of vehicular accidents, and from knife and gunshot wounds. Means to stop potentially fatal hemorrhage outside of the hospital environment is desirable.

Numerous hemostatic agents, compositions, and devices are known. Many such agents and compositions employ naturally procoagulant biopolymers, such as clotting factors and connective tissue proteins that have known hemostatic roles in vivo. Devices may include an inflatable member for blocking an opening in an artery, at which site a hemostatic agent may be introduced; other devices comprise agents or compositions in a particular form for application to a hemorrhaging site or plugging a puncture-type wound. For example, U.S. Patent 5,951,583 describes lyophilized thrombin, reconstituted with buffer and mixed with collagen, to form a viscous mixture. U.S. Patent 4,891,359 describes a hemostatic collagen paste composition, which optionally includes thrombin. U.S. Patent 5,595,735 described a hemostatic composition comprising thrombin in a polyethylene glycol base. U.S. Patent 5,948,425 described a hemostatic plug comprising collagen for sealing an incision which comprises a bleeding blood

vessel. These and all other citations herein are incorporated herein by reference in their entireties.

Certain metal ions have known hemostatic properties. The use of silver nitrate as a topically applied cautery agent is known. As described in copending application Serial No. 09/022,449, filed February 12, 1998, now U.S. patent 6,245,573, metal ions such as mercuric ion, silver ion, ion, cadmium ion, copper ion, barium ion, tin ion, selenate ion and tungstate ion are useful to modulate the clotting rate of blood in order to diagnose coagulopathies. Silver metal, in the form of colloidal silver, has been used as an antimicrobial agent for many years, predating the use of antibiotics, but still used today particularly in the management of burn patients.

It is toward the development of improved hemostatic compositions and devices that the present invention is directed.

The citation of any reference herein should not be construed as an admission that such reference is available as "Prior Art" to the instant application.

SUMMARY OF THE INVENTION

In its broadest aspect, the present invention is directed to a hemostatic composition which comprises at least one procoagulant metal ion and at least one procoagulant biopolymer. The metal ion may be, by way of non-limiting example, silver (I) or mercury (II). Preferably, silver (I) ion is used. Such ions, particularly silver, may be used at very low concentrations, for example, as low as the level of free silver ion available from an insoluble silver salt such as silver chloride. The procoagulant biopolymer may be, for example, one or more of the following: collagen, thrombin, prothrombin, fibrin, fibrinogen, heparinase, Factor VIIa, Factor VIII, Factor IXa, Factor Xa, Factor XII, von Willebrand Factor, a selectin, a procoagulant venom, a plasminogen activator inhibitor, glycoprotein IIb-IIIa, proteases, or plasma. The hemostatic composition of the invention may also include a carrier, such as, but not limited to, polyethylene glycol, hyaluronic acid, methyl cellulose, or albumin. In a preferred embodiment, the concentration of the metal ion present in the biopolymer may be reduced below its effective hemostatic concentration in the absence of the biopolymer, as the instant inventors have found surprisingly a synergy between the procoagulant biopolymer and the procoagulant metal ion. In another embodiment, the level of the procoagulant biopolymer may be reduced well below its

effective hemostatic concentration in the absence of the metal ion. In a further embodiment, the hemostatic composition comprises a procoagulant polymer at a sub-coagulant level in the absence of a metal ion, in combination with a metal ion at a sub-coagulant level in the absence of a procoagulant polymer.

The hemostatic composition of the invention may be in the form of, for example, a paste, dough, glue, liquid, lyophilized powder or foam, for application to a wound.

In another aspect, the invention is directed to a hemostatic device which comprises a hemostatic composition as described above. The device may be in the form of, for example, a plug, bandage, gauze, cloth, tampon, membrane or sponge.

A method for the prophylaxis or treatment of bleeding at a site is provided herein by applying to the site a hemostatic composition as described above, or a hemostatic device as described above. In one embodiment, the methods and compositions of the invention are useful in treating hemorrhaging, hypothermic patients.

The present invention is further directed to a method for the prophylaxis or treatment of bleeding at a site by applying to the site a composition comprising at least one procoagulant metal ion and at least one procoagulant biopolymer. The procoagulant metal ions and biopolymers are as described hereinabove. The composition may include a carrier, as described above. The hemostatic composition of the invention may be in the form of, for example, a paste, dough, glue, liquid, lyophilized powder or foam. Other forms of the composition are embraced herein.

In another aspect, the invention is directed to a method for the prophylaxis or treatment of bleeding at a site by applying to the site a device comprising a composition comprising at least one procoagulant metal ion and at least one procoagulant biopolymer. The procoagulant metal ions and procoagulant biopolymers are as described hereinabove. As noted above, the device may be in the form of a plug, bandage, gauze, cloth, tampon, membrane or sponge. Other forms of the device are embraced herein.

These and other aspects of the present invention will be better appreciated by reference to the following drawings and Detailed Description.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the effect of various concentrations of silver (I) ion on clotting of whole blood and plasma.

Figure 2 shows the effect of collagen on clotting of whole blood.

Figure 3 shows the effect of silver (I) ion and collagen on the clotting of heparinized whole blood.

Figure 4 shows the effect of silver (I) ion and collagen on the clotting of heparinized plasma.

Figure 5 shows further the effect of silver (I) ion and collagen on the clotting of heparinized plasma.

Figure 6 shows further the effect of silver (I) ion and collagen on clotting of whole blood.

Figure 7 shows the effect of silver (I) and collagen on clotting time of whole blood with low molecular weight heparin.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is broadly directed to hemostatic compositions and devices comprising at least one procoagulant metal ion and at least one procoagulant biopolymer, optionally with a carrier. Although silver (I) ion has been used as a cautery agent to stop bleeding by direct application of a soluble silver salt such as silver nitrate to a wound, it was found herein by surprise that an improved hemostatic composition was preparable using the combination of at least one procoagulant metal ion, such as silver (I) ion, and at least one procoagulant biopolymer, such as collagen. Furthermore, the inventor has found that the combination of a low concentrations of a procoagulant metal ion and a procoagulant biopolymer provides an effective hemostatic composition at concentrations wherein the individual components alone are less or not at all effective. For example, the concentration of free silver ion available in a composition of the invention comprising what is generally regarded as the water-insoluble silver salt silver

chloride is adequate to promote hemostasis in combination with a procoagulant polymer. Thus, a composition of the present invention achieves the desired hemostatic activity with low concentrations of components, offering less potential toxicity, more rapid biodegradation, lower cost of raw materials, and superior activity in wound dressings and arterial plugs. A further advantage is that the clot formed in the presence of the instant compositions are stronger than clots formed in the absence of the compositions.

The inventor has found that the present procoagulant compositions are useful for inducing clotting in both whole blood and in plasma. As will be seen below, the procoagulant properties of the present compositions are also useful in the presence of anticoagulant factors such as heparin. As patients undergoing anticoagulant therapy are prone to prolonged and thus dangerous bleeding, the present compositions are particularly useful for use in surgery or trauma care in such patients.

The invention has been described in co-pending provisional application serial no. 60/212,229, filed June 16, 2000, and is incorporated herein by reference in its entirety. Moreover, priority of the present application is claimed to the aforementioned provisional application under 35 U.S.C. § 119(e). As shown therein, various metal ions at low concentrations in combination with a procoagulant biopolymer were found to act synergistically to promote coagulation or clotting of whole blood.

Further studies herein have confirmed and expanded the scope of the invention. Measurements of the procoagulant effects of the compositions of the invention have been carried out using a SONOCLOT miniviscometer to detect early clot formation. In such studies, anti-coagulated, citrated whole blood is recalcified to initiate the clotting process in a device which sensitively monitors the formation of the clot. Studies have been expanded from whole blood to include plasma.

Various metal ions known to have procoagulant properties are useful in the compositions and devices of the present invention. The present invention embraces all such procoagulant metal ions. These ions are set forth herein as the element followed by the number of positive charges of the ion in parentheses, such as silver (I) or mercury (II) [also known as mercuric]. Any salts of these ions are suitable for use intended herein that provide a effective procoagulant level of free metal ion. Using silver (I) as an example, the nitrate salt provides adequate free silver (I) ion,

whereas the chloride salt provides less, being less soluble and with a low dissociation constant. However, as mentioned above, certain metal salts which are considered insoluble have a large-enough dissociation constant to permit an effective procoagulant level of the metal ion to be present in the compositions herein which comprise the insoluble metal salt. The skilled artisan will be able to readily determine the suitable salt form of the metal ion that provides the procoagulant properties in combination with the procoagulant biopolymer. Furthermore, the skilled artisan will be aware of the compatibility of the salt forms of the metal(s) and other components of the composition to maintain adequate levels of the metal ion(s) in solution to provide procoagulant activity. Of course, considerations must be given to the toxicity of the metal salt, whether soluble or not, at the concentrations needed for an effective hemostatic composition.

As noted herein, the composition may comprise one or more procoagulant metal ions, such as silver (I) or mercury (II). For the example of silver (I) ion, effective levels may be achieved by using water-soluble silver salts such as the nitrate, selenate, nitrite, or perchlorate salts, or organic acids such as lactate. In the case of mercury (II), nitrate, acetate and chloride salts are examples. Mercury (II) ion in the form of MERCUROCHROME(R) (2',7'-dibromo-5-[hydroxymercuric]fluorescein) may also be used. As noted above, when such salts are used in combination, one must be cognizant of the compatibilities of the salts to avoid reducing the solubility of the procoagulant ion(s) below their effective concentration(s). Furthermore, certain components may provide oxidative or reductive properties which in contact with the metal ion(s), may alter their oxidation state and effectiveness. Such considerations are well within the realm of the skilled artisan in preparing an effective procoagulant composition in accordance with the disclosure herein.

As noted above, a composition of the present invention may have more than one metal salt, such as the combination of silver (I) and mercury (II).

With regard to the procoagulant biopolymer of the instant composition, this component embraces naturally occurring, recombinant, isolated, and any and all other forms of procoagulant proteins and other biologically compatible polymers with procoagulant activity. By way of non-limiting example, procoagulant proteins embraced herein include but are not limited to collagen, thrombin, prothrombin, fibrin, fibrinogen, heparinase, Factor VIIa, Factor VIII, Factor IXa, Factor Xa, Factor XII, von Willebrand Factor, a selectin, a procoagulant venom, a plasminogen

activator inhibitor, glycoprotein IIb-IIIa, proteases, or plasma. These may be used singly or in combination. Such proteins include those which have a net effect of procoagulant activity, such as clot dissolution inhibitors including plasminogen activator inhibitor. Included herein is plasma, which comprises numerous procoagulant proteins, and proteases, which are procoagulant. An example of a procoagulant venom is Russells' viper venom. Examples of proteases are described in the catalog of Sigma Chemical Company, from numerous sources.

The procoagulant proteins of the invention may be prepared by any of numerous methods or obtained from commercial sources. Many such proteins are available in purified form, for example, from Sigma Chemical Co., American Diagnostica, and Critichem Inc., to name only a few examples.

A preferred procoagulant polymer is collagen, and more preferred, type I collagen. It is available from any of a number of sources in various forms, including fibrillar, amorphous, and others. Particular preparations used for certain of the studies herein are sponge collagens, woven collagens and fibrillar collagens from the Collagen Products Division of Datascope Corp., Mahwah, New Jersey, U.S.A.

The concentrations of the procoagulant metal ion(s) and procoagulant biopolymer(s) in the composition of the invention may be prepared in accordance with the desired properties of the composition, the form of the device in which the composition is delivered or applied to the wound, and other factors that one or ordinary skill in the art would take into account in preparing the composition or device of the invention. Of course, the composition must have low toxicity, and if left in place, preferably needs to biodegrade after it has achieved its desired function. As noted above, it was found that the concentration of the procoagulant metal ion(s) in the instant composition may be reduced to a level below which it would not be effective as the sole hemostatic agent. For example, for a particular sample of patient blood, a composition comprising silver (I) and collagen may comprise 1% collagen and 0.1% silver nitrate, wherein 0.1% silver nitrate alone would not impart significant hemostasis in a wound. Therefore, the hemostatic compositions of the present invention may, in one embodiment, include either the procoagulant biopolymer or the procoagulant metal ion, or both, at a concentration lower than would be hemostatically effective alone.

Examples of suitable compositions of the invention comprising one procoagulant biopolymer include, but are not limited to, silver (I) and collagen, silver (I) and thrombin, silver (I) and prothrombin, silver (I) and fibrin, silver (I) and fibrinogen, silver (I) and heparinase, silver (I) and Factor VIIa, silver (I) and Factor VIII, silver (I) and Factor IXa, silver (I) and Factor Xa, silver (I) and Factor XII, silver (I) and von Willebrand Factor, silver (I) and a selectin, silver (I) and a procoagulant venom, silver (I) and a plasminogen activator inhibitor, silver (I) and glycoprotein IIb-IIIa, silver (I) and a protease, silver (I) and plasma; mercury (II) and collagen, mercury (II) and thrombin, mercury (II) and prothrombin, mercury (II) and fibrin, mercury (II) and fibrinogen, mercury (II) and heparinase, mercury (II) and Factor VIIa, mercury (II) and Factor VIII, mercury (II) and Factor IXa, mercury (II) and Factor Xa, mercury (II) and Factor XII, mercury (II) and von Willebrand Factor, mercury (II) and a selectin, mercury (II) and a procoagulant venom, mercury (II) and a plasminogen activator inhibitor, mercury (II) and glycoprotein IIb-IIIa, mercury (II) and a protease, mercury (II) and plasma.

Preferred compositions include silver (I) and collagen, and mercury (II) and collagen. A preferred collagen is type I collagen. The collagen may be derived from any source, preferably mammalian and most preferably bovine in origin, but is it not so limiting. Human collagen, and in particular recombinantly-prepared human collagen, is another preferred procoagulant biopolymer.

Of course, the composition may contain a second or additional procoagulant metal ion, or additional procoagulant biopolymer(s). The invention embraces any combination of additional procoagulant metal ions or procoagulant polymers, as well as other excipients, carriers, stabilizers, preservatives and other additives to a hemostatic product normally needed to ensure stability, shelf life, and other commercial consideration. The known bacteriostatic activity of metal ions may provide an endogenous level of preservation in the compositions of the invention; moreover, the bacteriostatic properties are an additional benefit of the present compositions in use in wound care.

Thus; other hemostatic compositions of the invention include, but are not limited to, silver (I) and mercury (II) and collagen, silver (I) and mercury (II) and thrombin, silver (I) and mercury (II) and prothrombin, silver (I) and mercury (II) and fibrin, silver (I) and mercury (II) and fibrinogen, silver (I) and mercury (II) and heparinase, silver (I) and mercury (II) and Factor VIIa, silver (I) and mercury (II) and Factor VIII, silver (I) and mercury (II) and Factor IXa, silver (I) and

mercury (II) and Factor Xa, silver (I) and mercury (II) and Factor XII, silver (I) and mercury (II) and von Willebrand Factor, silver (I) and mercury (II) and a selectin, silver (I) and mercury (II) and a procoagulant venom, silver (I) and mercury (II) and a plasminogen activator inhibitor, silver (I) and mercury (II) and glycoprotein IIb-IIIa, silver (I) and mercury (II) and a protease, silver (I) and mercury (II) and plasma.

The hemostatic composition of the invention may also include a carrier, such as, but not limited to, polyethylene glycol, hyaluronic acid, cellulose, oxidized cellulose, methyl cellulose, or albumin. These may be used to provide a matrix, a suitable viscosity, deliverability, adherence, or other properties desired to be imparted to the compositions herein for easy in application to a wound. Numerous other carrier which impart these characteristics are embraced herein.

The form of the hemostatic composition of the invention may be prepared in any form suitable for use in the intended application. Various carriers and other materials may be provided in the composition to achieve the desired form, in addition to the at least one procoagulant metal ion and at least one procoagulant biopolymer. For example, the composition in the form of a paste may be prepared for application to a surgical site; a thicker paste, or dough, may be molded by the surgeon for application at a site of the removal of a transarterial cannula. A glue may be prepared with hemostatic properties; other forms include a liquid, a lyophilized powder or foam, for application to or in a wound. As will be described in more detail below in reference to devices comprising the hemostatic composition of the invention, the composition may be dried or lyophilized for form a particular shape, such as a plug, that is useful for particular applications, such as placement to stop the bleeding of a removed transarterial cannula. A bandage comprising a composition of the invention may also be provided. All of these various forms and other are embraced by the present invention.

As mentioned above, the invention is also directed to a hemostatic device which comprises a hemostatic composition as described above. The device may be provided in any number of formats useful for the control of bleeding. For example, the composition of the invention may be provided in the form of a plug, for placement in a wound; or provided on a bandage, gauze, cloth, tampon, membrane or sponge, for application to or placement in a wound. Certain materials may be biodegradable and suitable for placement in a closed surgical wound. Other materials may be provided for later removal. Devices for emergency application to hemorrhaging areas or severed arteries may be useful for emergency use.

In another embodiment of the invention, the hemostatic device comprising the composition may be prepared in an asymmetric fashion, of contain components asymmetrically applied, such that blood is restricted from flowing completely through a device. For example, a blood flow altering agent may be provided at the distal part of the device from that to which is applied to the wound, such that blood will not penetrate beyond the device. A bandage may comprise the instant composition on one side, and a blood- impermeant membrane on the other.

The present invention also embraces various methods for the prophylaxis or treatment of bleeding at a site in the body. This method is carried out by the application or placement of a hemostatic composition or a hemostatic device comprising the hemostatic composition of the invention to or in the site. The present invention embraced various methods and means used for the delivery or placement of the device in the site of desired hemostatic activity. The various forms of the composition and of the device are embraced within this method.

The present invention may be better understood by reference to the following non-limiting Examples, which are provided as exemplary of the invention. The following examples are presented in order to more fully illustrate the preferred embodiments of the invention. They should in no way be construed, however, as limiting the broad scope of the invention.

Example I

Effect of silver (I) ion and heparinase on clotting time of heparinized blood

The hemostatic compositions of the invention were evaluated in two different systems. In one system, clotting time of recalcified, citrated whole blood in the presence of the hemostatic composition was determined using a SONOCLOT Coagulation Analyzer (Sienco Inc., Wheat Ridge CO). Other instrumentation for providing clotting data are equally useful. Results were expressed as clotting time in seconds. In a second system, the hemostatic composition was dried on a cellulose matrix (filter paper), and 7µl aliquot of fresh blood or recalcified citrated whole blood was placed in the cellulose matrix; resulting in the spreading (perfusion) of the applied blood as an enlarging disk. Upon clotting or reduced perfusion, the further spread of the blood ceases. The surface area of the spread blood in mm^2 is used as an indication of the clotting rate of the blood. Results are expressed as mean ± standard deviation. The number of samples run is indicated at the top of each data column.

Silver (I) ion was provided as silver nitrate, 5% stock solution. It was diluted 100 fold to 0.05%, or in some cases to 0.01% in the clotting studies. In perfusion studies, a 10µl aliquot of the solution was applied to the filter paper then dried.

Collagen was from Sigma Chemical Co. In the clotting time studies, the final concentration of collagen was 0.1-0.2%. Heparinase was from Haemoscope Corp. and used at a final concentration of 1 U/mL.

Citrated blood anticoagulated with heparin (2 U/mL) was used as a model of the utility of the compositions of the present invention to induce hemostasis in an individual treated with anticoagulants (including fibrinolytics and antiplatelet therapies) as may occur in patients with myocardial infarction, stroke, unstable angina, thrombophlebitis, peripheral vascular disease, traumatic injury, post-surgical patients and those undergoing cancer chemotherapy. Recalcification time was measured.

Treatment	Clotting time (sec) (n=5)
Control	287 ± 21
Control + silver(I)	94 ± 35
Control + heparin	1930 ± 83
Control + heparin + heparinase	360 ± 22
Control + silver (I) + heparinase	872 ± 353
Control + silver (I) + heparin + heparinase	157 ± 30

These data show that silver (I) ion is capable of returning the recalcification time of blood towards normal; moreover, in combination with heparinase, is capable of reducing the clotting time to below normal values.

Example II

Effect of silver (I) ion and collagen on blood perfusion

Recalcified, citrated whole blood was applied to filter paper impregnated with 5% $AgNO_3$, 2% collagen, or the combination of 2.5% silver nitrate and 1% collagen. The size of the blood disk on the paper was measured.

Study I

Treatment	Perfusion (mm) (n=13)
5% $AgNO_3$	18 ± 4
2% collagen	35 ± 23
2.5%$AgNO_3$ + 1% collagen	7 ± 4

Study II

Treatment	Perfusion (mm) (n=5)
5% $AgNO_3$	17 ± 2
1% $AgNO_3$	42 ± 18
2% collagen	28 ± 10
2.5% $AgNO_3$ + 1% collagen	7 ± 0.4
0.5% $AgNO_3$ + 1% collagen	15 ± 5

This study shows the effectiveness of the combination of $AgNO_3$ and collagen, and furthermore, that the combination of a lower concentration of silver (I) ion, 0.5%, with collagen (1%) can achieve the hemostatic effect provided by 5% $AgNO_3$ alone.

Example IV

Effect of silver (I) ion and collagen on clotting time

The final concentrations of the composition in blood is provided below for a similar experiment.

Treatment	Clotting time (sec) [n=4]
Control	273 ± 46
Control + 1% collagen	264 ± 37
Control + 0.6 mM $AgNO_3$	220 ± 50
Control + 0.3 mM $AgNO_3$ + 0.5% collagen	178 ± 42

This study demonstrates the combined effect of silver (I) ion and collagen on hemostatic measured by clotting time of recalcified, citrated whole blood.

Example V

Effect of silver (I) ion on clotting time of whole blood, platelet-rich blood, and platelet-poor blood

This study was undertaken using platelet-rich and platelet-poor plasma to simulate an individual with normal and reduced platelet levels.

Treatment	Clotting time (sec) [n=8]
Normal Blood	262 ± 26
Normal blood + 2.9 mM $AgNO_3$	109 ± 20
Platelet Rich Plasma (PRP)	314 ± 70
PRP + 2.9 mM $AgNO_3$	83 ± 29
Platelet Poor Plasma (PPP)	353 ± 62
PPP + 2.9 mM $AgNO_3$	128 ± 32

These studies show the effectiveness of silver (I) ion as a hemostatic agent in blood with low platelet numbers.

Example VI

Clot strength after silver (I) ion induced clotting of heparinized blood

Using the SONOCLOT, tracings of heparinized whole blood clotting in the presence of silver (I) ion were obtained. While the clotting time of heparinized blood is shortened from 1930 seconds to 872 seconds in the presence of silver (I) ion, the physical characteristics of the silver-induced clot as shown by the analyzer demonstrates a stronger clot. In non-heparinized blood, the clotting time is shortened from 217 seconds to 81 seconds in the presence of silver; furthermore, the clot is stronger. In a second study, silver (I) ion reduces the clotting time from 588 to 168 seconds, also with a stronger clot.

Example VI

Silver (I) ion and thrombin potentiates clotting time compared to either alone

Thrombin was used at a final concentration of 0.025 U/mL. Silver ion was present at a final concentration of 2.9 mM.

Treatment	Clotting time (sec) [n=8]
Control	236 ± 38
Control + thrombin	156 ± 42
Control + silver	91 ± 14
Control + silver + thrombin	71 ± 7

Example VII

Effect of various concentrations of silver (I) ion on clotting of whole blood and plasma

Figure 1 depicts the clotting time of citrated whole blood (A-D) and plasma (E-H) in the presence of 0.05% silver (I) ion (B, F), 0.005% silver (I) ion (C, G), 0.0005% silver (I) ion (E, H), or no silver ion (A, E).

Example VIII

Effect of collagen preparations and silver (I) ion on clotting of whole blood

Figure 2 depicts the clotting times of whole blood alone (A) or in the presence of 1 mg/ml sponge collagen (B), 1 mg/ml fibrillar collagen (C), 0.05% silver (I) ion (D), 0.05% silver (I) ion and 1 mg/ml sponge collagen (E), and 0.05% silver (I) ion and 1 mg/ml fibrillar collagen (F).

Example IX

Effect of collagen and silver ion on clotting of heparinized whole blood

Figure 3 shows the clotting times of heparinized whole blood (1 U/ml) alone (A) or in combination with 0.05% silver (I) ion (B), with 1 mg/ml fibrillar collagen (C), and in the presence of the combination of 0.05% silver (I) ion and 1 mg/ml sponge collagen (D). Bar E shows the clotting time of the heparinized whole blood with 1 mg/ml sponge collagen; and in combination with 0.05% silver (I) ion in (F).

Figure 4 similarly shows the effect on heparinized whole blood clotting time in the presence of 0.05% silver (I) ion, collagen and 0.25 U/ml heparin (A), and under similar conditions with 0.3% silver (I) ion (B). However, in the absence of collagen at either silver (I) levels, a prolonged clotting time is seen C and D, respectively.

Figure 5 shows the clotting time of heparinized whole blood (1 U/ml) with various concentrations of silver ion or mercuric ion. Heparinized whole blood alone (A), with sponge collagen (B), with sponge collagen and 0.05% silver (I) ion (C), with sponge collagen and 0.3% silver (I) ion (D), or with sponge collagen and 0.1% mercuric (II) ion (E). The same series with fibrillar collagen are shown in F-I, respectively.

Figure 6 shows the clotting time of heparinized whole blood (2U/ml): whole blood (A), heparinized whole blood (B), activated clotting time of heparinized whole blood (C), clotting time of heparinized whole blood plus fibrous collagen (D), or sponge collagen (E); the clotting time of heparinized whole blood with fibrous collagen and 0.05% silver (I) ion (F), the same with 0.5% silver (I) ion (G), or sponge collagen collagen with 0.05% silver (I) ion (H) or 0.5% silver (I) ion (I). Heparinized whole blood with 0.05% silver (I) ion is shown in J, and with 0.5% silver (I) ion in K.

Figure 7 shows the effect of low molecular weight (LMW) heparin and fibrous collagen on clotting time of whole blood. LMW heparinized whole blood (A), with 0.05% silver (I) ion (B), with 0.3% silver (I) ion (C), LMW heparinized whole blood plus fibrous collagen (D), the same plus 0.05% silver (I) ion (E), or with 0.5% silver (I) ion (F).

The foregoing studies demonstrate the effect of the combination of a procoagulant polymer, sponge (cross-linked) or fibrous collagen, and silver (I) or mercury (II) ion, on promoting coagulation of plasma, whole blood, and heparinized plasma and whole blood.

The present invention is not to be limited in scope by the specific embodiments describe herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

WHAT IS CLAIMED IS:

1. A hemostatic composition comprising at least one procoagulant biopolymer in combination with a procoagulant metal ion, said procoagulant metal ion present in said composition at a level below its effective hemostatic concentration in the absence of said procoagulant biopolymer.

2. The composition of claim 1 wherein said procoagulant metal ion is selected from the group consisting of silver (I) and mercury (II).

3. The composition of claim 2 wherein said procoagulant metal ion is silver (I).

4. The composition of claim 2 wherein said procoagulant metal ion is provided by an insoluble salt comprising said metal ion.

5. The composition of claim 4 wherein said procoagulant metal ion is silver (I).

6. The composition of claim 5 wherein said salt is silver chloride.

7. The composition of claim 1 wherein said at least one procoagulant biopolymer is selected from the group consisting of collagen, thrombin, prothrombin, fibrin, fibrinogen, heparinase, Factor VIIa, Factor VIII, Factor IXa, Factor Xa, Factor XII, von Willebrand Factor, a selectin, a procoagulant venom, a plasminogen activator inhibitor, glycoprotein IIb-IIIa, a protease, and plasma.

8. The composition of claim 7 wherein said at least one procoagulant biopolymer is collagen.

9. The hemostatic composition of claim 1 selected from the group consisting of silver (I) and collagen, silver (I) and thrombin, silver (I) and prothrombin, silver (I) and fibrin, silver (I) and fibrinogen, silver (I) and heparinase, silver (I) and Factor VIIa, silver (I) and Factor VIII, silver (I) and Factor IXa, silver (I) and Factor Xa, silver (I) and Factor XII, silver (I) and von Willebrand Factor, silver (I) and a selectin, silver (I) and a procoagulant venom, silver (I) and a plasminogen activator inhibitor, silver (I) and glycoprotein IIb-

IIIa, silver (I) and a protease, silver (I) and plasma, mercury (II) and collagen, mercury (II) and thrombin, mercury (II) and prothrombin, mercury (II) and fibrin, mercury (II) and fibrinogen, mercury (II) and heparinase, mercury (II) and Factor VIIa, mercury (II) and Factor VIII, mercury (II) and Factor IXa, mercury (II) and Factor Xa, mercury (II) and Factor XII, mercury (II) and von Willebrand Factor, mercury (II) and a selectin, mercury (II) and a procoagulant venom, mercury (II) and a plasminogen activator inhibitor, mercury (II) and glycoprotein IIb-IIIa, mercury (II) and a protease, and mercury (II) and plasma.

10. The composition of claim 1 further comprising a carrier.

11. The composition of claim 10 wherein said carrier is selected from the group consisting of polyethylene glycol, hyaluronic acid, cellulose, oxidized cellulose, methyl cellulose, and albumin.

12. The composition of claim 1 wherein said composition is in the form of a paste, glue, liquid, lyophilized powder or foam.

13. A hemostatic device comprising the hemostatic composition of claim 1.

14. The device of claim 13 comprising a plug, bandage, gauze, cloth, tampon, membrane or sponge.

15. A method for the prophylaxis or treatment of bleeding at a site comprising applying to said site the hemostatic composition of claim 1.

16. A method for the prophylaxis or treatment of bleeding at a site comprising applying to said site the device of claim 13.

17. A method for the prophylaxis or treatment of bleeding at a site comprising applying to said site a hemostatic composition comprising at least one procoagulant biopolymer in combination with a procoagulant metal ion, said procoagulant metal ion present in said composition at a level below its effective hemostatic concentration in the absence of said procoagulant biopolymer.

18. The method of claim 17 wherein said metal ion is selected from the group consisting of silver (I) or mercury (II).

19. The method of claim 17 wherein said metal ion is silver (I).

20. The method of claim 17 wherein said at least one procoagulant biopolymer is selected from the group consisting of collagen, thrombin, prothrombin, fibrin, fibrinogen, heparinase, Factor VIIa, Factor VIII, Factor IXa, Factor Xa, Factor XII, von Willebrand Factor, a selectin, a procoagulant venom, a plasminogen activator inhibitor, glycoprotein IIb-IIIa, a protease and plasma.

21. The method of claim 20 wherein said procoagulant biopolymer is collagen.

22. The method of claim 17 wherein said hemostatic composition is selected from the group consisting of silver (I) and collagen, silver (I) and thrombin, silver (I) and prothrombin, silver (I) and fibrin, silver (I) and fibrinogen, silver (I) and heparinase, silver (I) and Factor VIIa, silver (I) and Factor VIII, silver (I) and Factor IXa, silver (I) and Factor Xa, silver (I) and Factor XII, silver (I) and von Willebrand Factor, silver (I) and a selectin, silver (I) and a procoagulant venom, silver (I) and a plasminogen activator inhibitor, silver (I) and glycoprotein IIb-IIIa, silver (I) and a protease, silver (I) and plasma, mercury (II) and collagen, mercury (II) and thrombin, mercury (II) and prothrombin, mercury (II) and fibrin, mercury (II) and fibrinogen, mercury (II) and heparinase, mercury (II) and Factor VIIa, mercury (II) and Factor VIII, mercury (II) and Factor IXa, mercury (II) and Factor Xa, mercury (II) and Factor XII, mercury (II) and von Willebrand Factor, mercury (II) and a selectin, mercury (II) and a procoagulant venom, mercury (II) and a plasminogen activator inhibitor, mercury (II) and glycoprotein IIb-IIIa, mercury (II) and a protease, and mercury (II) and plasma.

24. The method of claim 17 wherein said hemostatic composition further comprises a carrier.

25. The method of claim 24 wherein said carrier is selected from the group consisting of polyethylene glycol, hyaluronic acid, cellulose, oxidized cellulose, methyl cellulose, and albumin.

26. The method of claim 17 wherein said composition is in the form of a paste, glue, liquid, lyophilized powder or foam.

27. A method for the prophylaxis or treatment of bleeding at a site comprising applying to said site a device comprising a hemostatic composition comprising at least one procoagulant biopolymer in combination with a procoagulant metal ion, said procoagulant metal ion present in said composition at a level below its effective hemostatic concentration in the absence of said procoagulant biopolymer.

28. The method of claim 27 wherein said metal ion is selected from the group consisting of silver (I) and mercury (II).

29. The method of claim 28 wherein said metal ion is provided by an insoluble salt comprising said metal ion.

30. The method of claim 29 wherein said metal ion is silver (I).

31. The method of claim 30 wherein said salt is silver chloride.

32. The method of claim 27 wherein said at least one procoagulant biopolymer is selected from the group consisting of collagen, thrombin, prothrombin, fibrin, fibrinogen, heparinase, Factor VIIa, Factor VIII, Factor IXa, Factor Xa, Factor XII, von Willebrand Factor, a selectin, a procoagulant venom, a plasminogen activator inhibitor, glycoprotein IIb-IIIa, a proteases and plasma.

33. The method of claim 32 wherein said procoagulant biopolymer is collagen.

34. The method of claim 27 wherein said hemostatic composition is selected from the group consisting of silver (I) and collagen, silver (I) and thrombin, silver (I) and prothrombin, silver (I) and fibrin, silver (I) and fibrinogen, silver (I) and heparinase, silver (I) and Factor VIIa, silver (I) and Factor VIII, silver (I) and Factor IXa, silver (I) and Factor Xa, silver (I) and Factor XII, silver (I) and von Willebrand Factor, silver (I) and a selectin, silver (I) and a procoagulant venom, silver (I) and a plasminogen activator inhibitor, silver (I) and glycoprotein IIb-IIIa, silver (I) and a protease, silver (I) and plasma, mercury

(II) and collagen, mercury (II) and thrombin, mercury (II) and prothrombin, mercury (II) and fibrin, mercury (II) and fibrinogen, mercury (II) and heparinase, mercury (II) and Factor VIIa, mercury (II) and Factor VIII, mercury (II) and Factor IXa, mercury (II) and Factor Xa, mercury (II) and Factor XII, mercury (II) and von Willebrand Factor, mercury (II) and a selectin, mercury (II) and a procoagulant venom, mercury (II) and a plasminogen activator inhibitor, mercury (II) and glycoprotein IIb-IIIa, mercury (II) and a protease, and mercury (II) and plasma.

35. A hemostatic composition comprising at least one procoagulant biopolymer in combination with a procoagulant metal ion, said procoagulant metal ion present in said composition at a level below its effective hemostatic concentration in the absence of said procoagulant biopolymer, and said procoagulant biopolymer present in said composition at a level below its effective hemostatic concentration in the absence of said procoagulant metal ion.

36. A method for the prophylaxis or treatment of bleeding at a site comprising applying to said site the hemostatic composition of claim 35.

FIG. 1

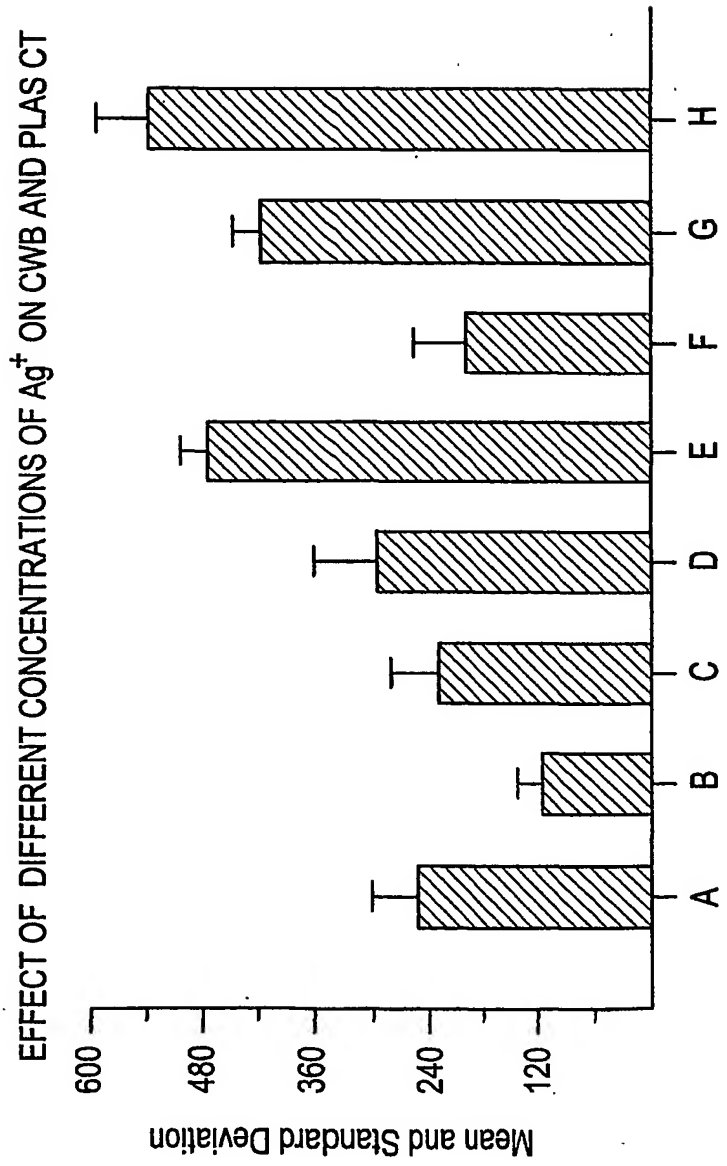

EFFECT OF DIFFERENT CONCENTRATIONS OF Ag+ ON CWB AND PLAS CT
Mean and Standard Deviation
600
480
360
240
120
A
B
C
D
E
F
G
H

FIG. 2

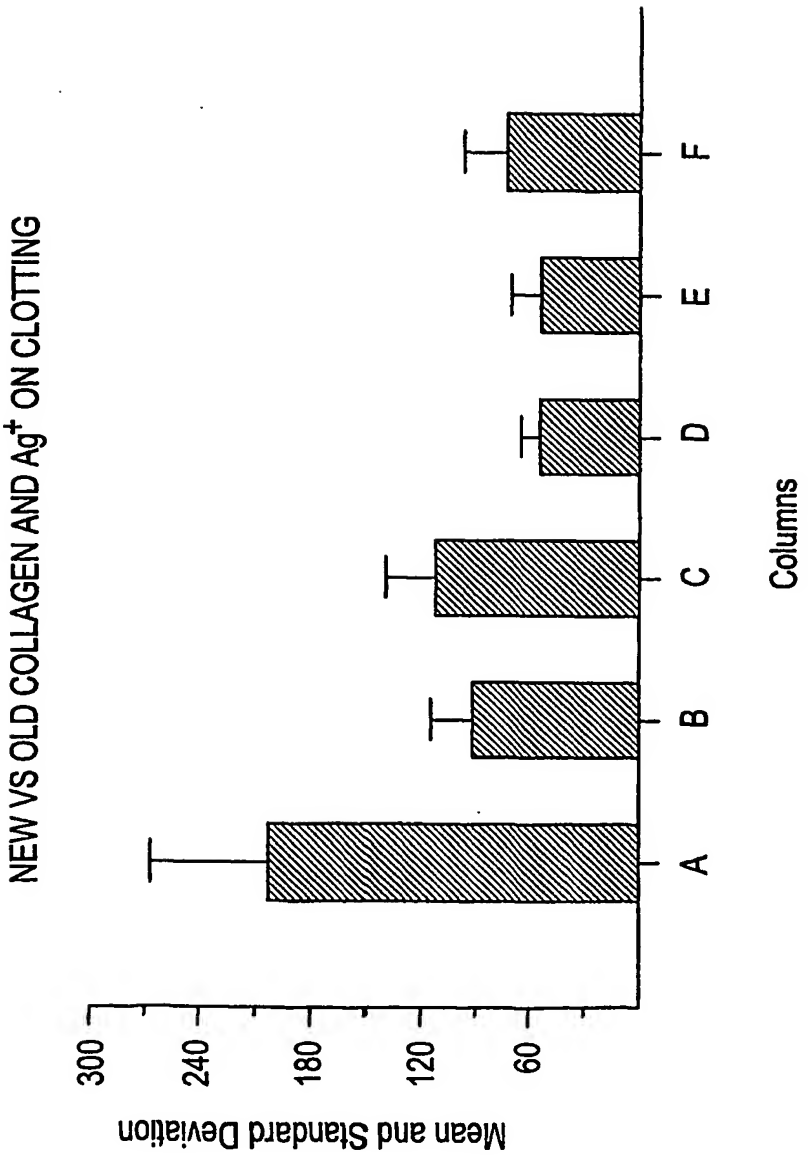

NEW VS OLD COLLAGEN AND Ag+ ON CLOTTING
Mean and Standard Deviation
300
240
180
120
60
A
B
C
D
E
F
Columns

FIG. 3

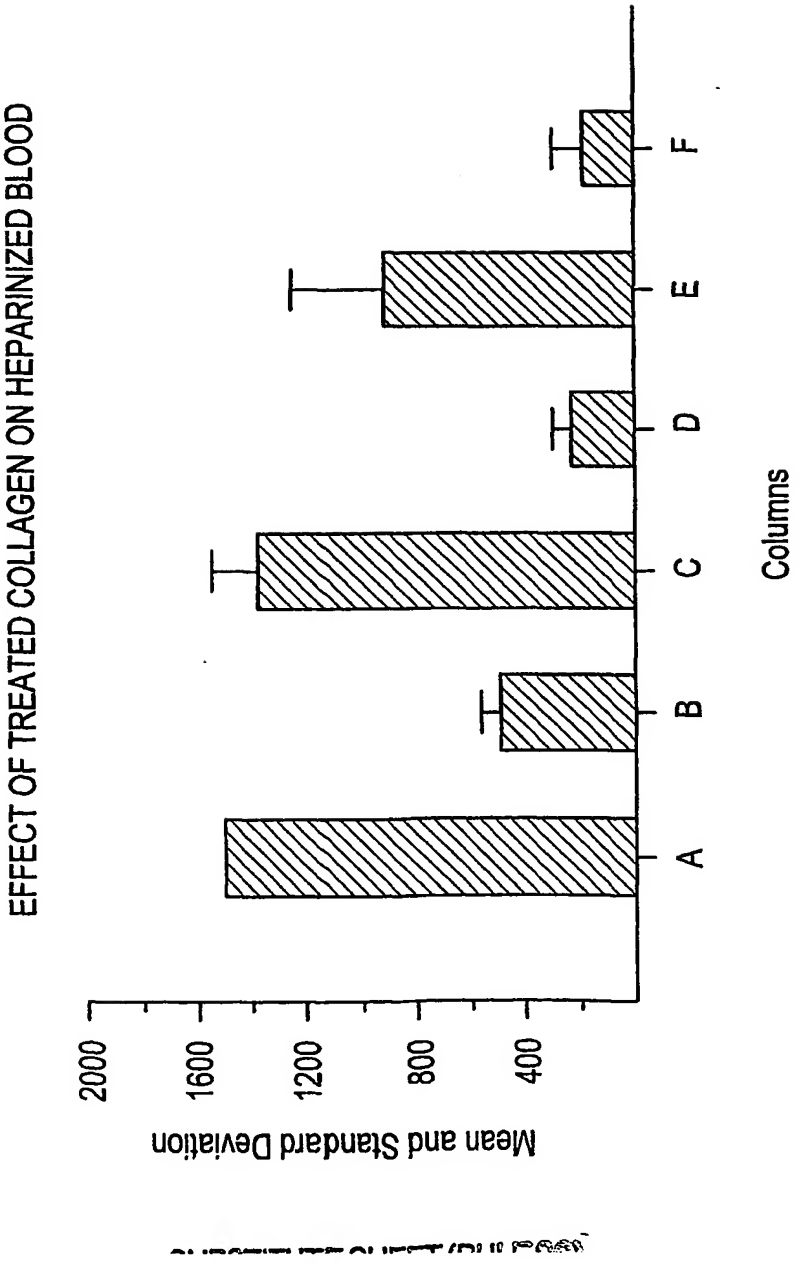

EFFECT OF TREATED COLLAGEN ON HEPARINIZED BLOOD
Mean and Standard Deviation
2000
1600
1200
800
400
A
B
C
D
E
F
Columns

FIG.4

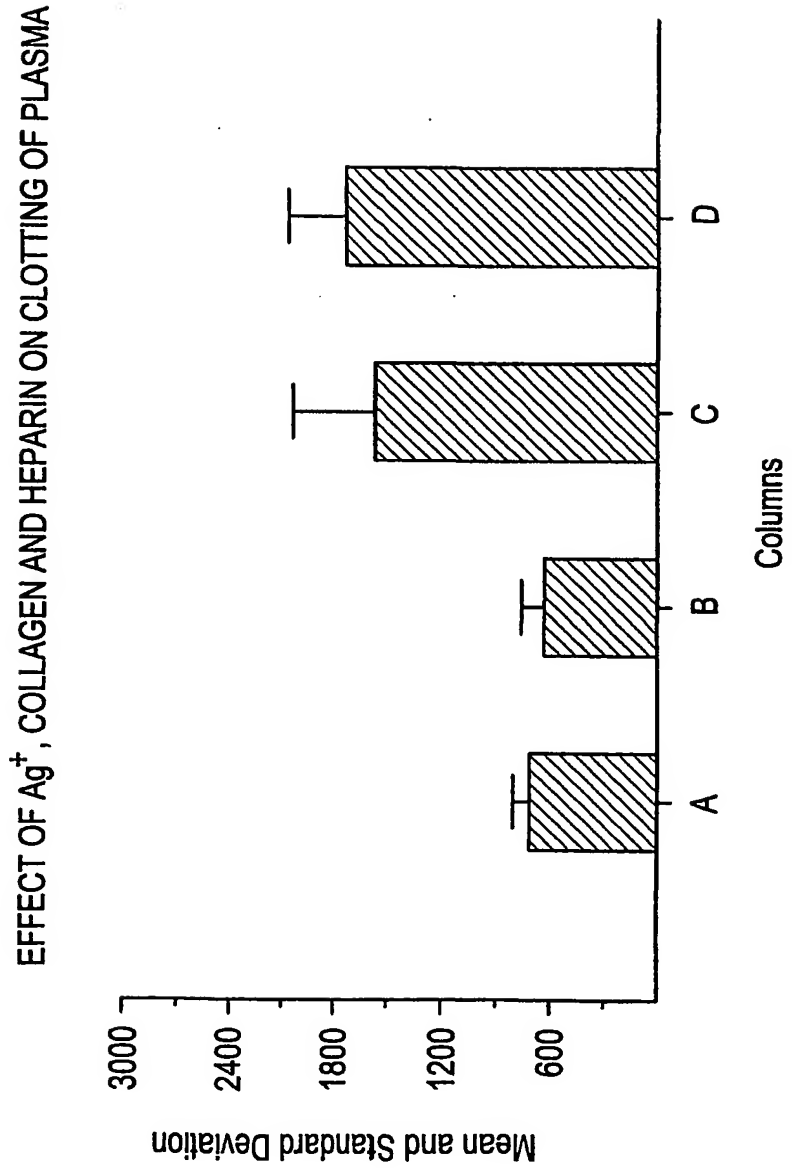

EFFECT OF Ag+, COLLAGEN AND HEPARIN ON CLOTTING OF PLASMA
Mean and Standard Deviation
3000
2400
1800
1200
600
A
B
C
D
Columns

FIG. 5

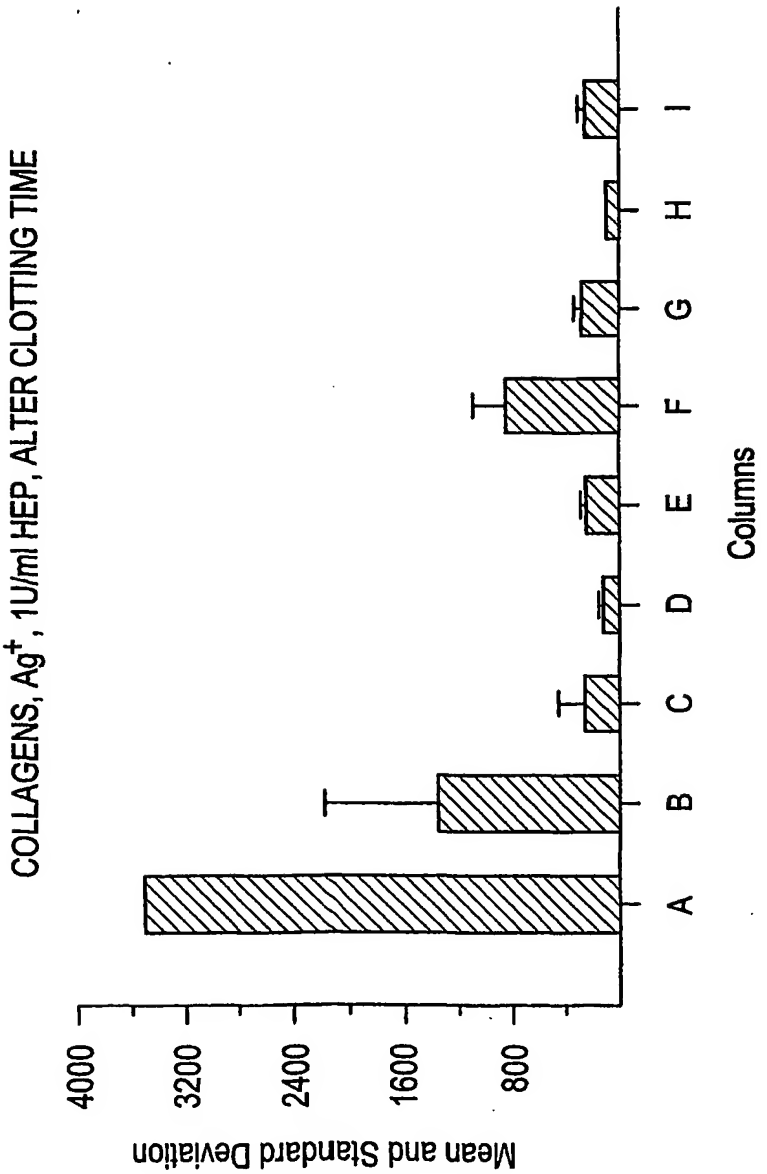

COLLAGENS, Ag+, 1U/ml HEP, ALTER CLOTTING TIME
Mean and Standard Deviation
4000
3200
2400
1600
800
A
B
C
D
E
F
G
H
I
Columns

FIG. 6

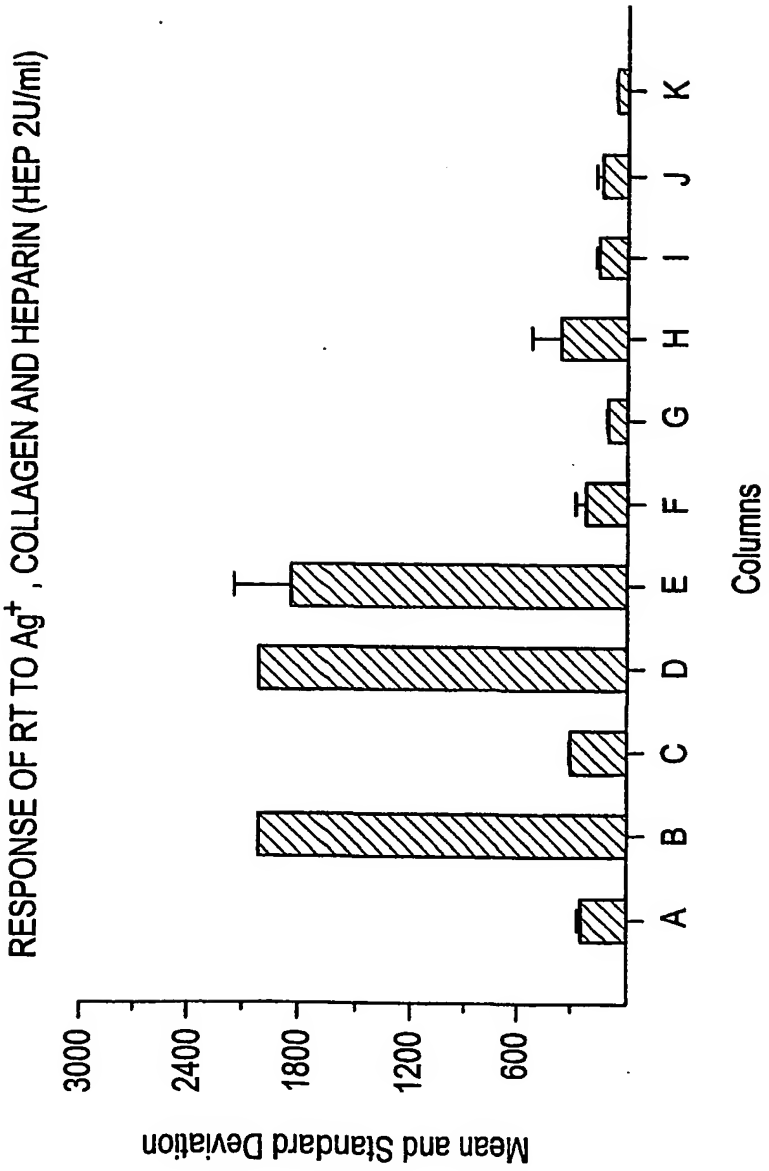
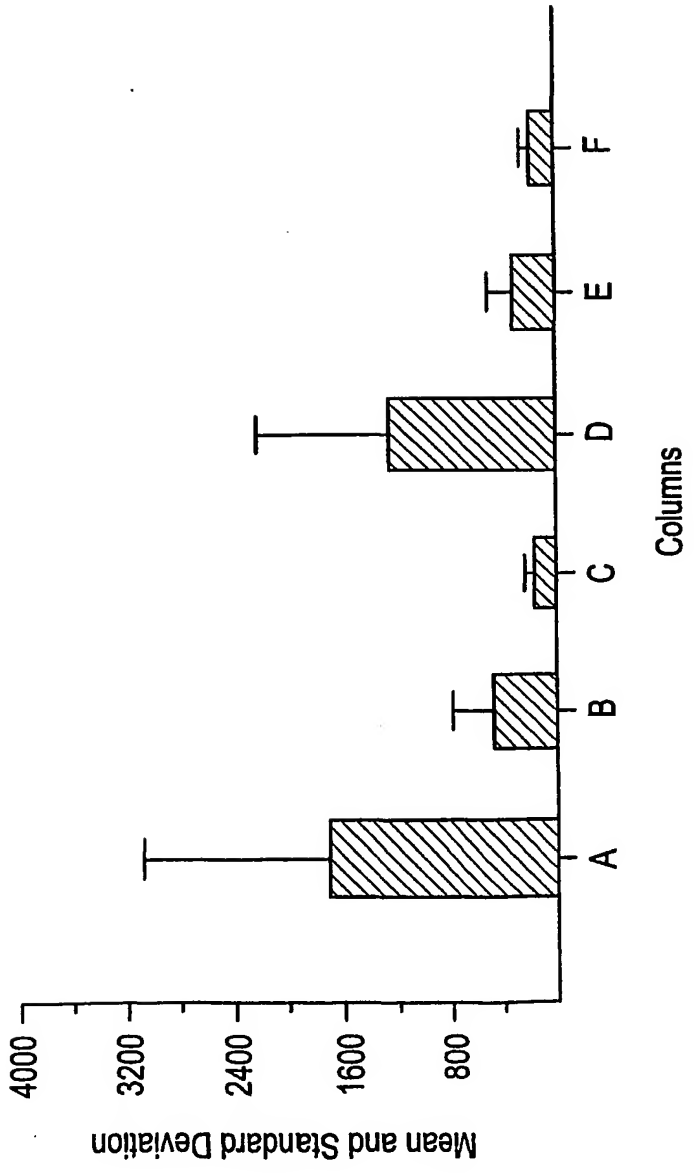

RESPONSE OF RT TO Ag+ , COLLAGEN AND HEPARIN (HEP 2U/ml)
Mean and Standard Deviation
3000
2400
1800
1200
600
A
B
C
D
E
F
G
H
I
J
K
Columns

FIG. 7


EFFECT OF LMWH ON NEW COLLAGEN AND Ag+
Mean and Standard Deviation
4000
3200
2400
1600
800
A
B
C
D
E
F
Columns

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(71) Applicant *(for all designated States except US)*: **UNIVERSITY OF MEDICINE AND DENTISTRY OF NEW JERSEY** [US/US]; P.O. Box 2688, New Brunswick, NJ 08903 (US).

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(74) Agent: YAMIN, Michael, A.; Klauber & Jackson, 411 Hackensack Avenue, Hackensack, NJ 07601 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report:
9 January 2003

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/097826 A3

(54) Title: HEMOSTATIC COMPOSITIONS, DEVICES AND METHODS

(57) Abstract: A hemostatic composition which comprises at least one procoagulant metal ion, such as silver (I) or mercury (II), and at least one procoagulant biopolymer, such as collagen, thrombin, prothrombin, fibrin, fibrinogen, heparinase, Factor VIIa, Factor VIII, Factor IXa, Factor Xa, Factor XII, von Willebrand Factor, a selectin, a procoagulant venom, a plasminogen activator inhibitor, glycoprotein IIb-IIIa, a protease, or plasma. The composition in the form of a paste, dough, glue, liquid, lyophilized powder or foam, may be provided, for application to a wound. A hemostatic device is also described which comprises a hemostatic composition as described above. The device may be in the form of, for example, a plug, bandage, gauze, cloth, tampon, membrane or sponge. Methods are also provided for prophylaxis or treatment of bleeding at a site by application to the site of the composition or device as described.

INTERNATIONAL SEARCH REPORT

	I ional Application No rc1/US 01/19145

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K38/39 A61K38/37 A61K38/36 A61K33/38 A61K33/28
A61P7/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
PAJ, EPO-Internal, WPI Data, BIOSIS, CHEM ABS Data, EMBASE, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 04828 A (RIVAROSSA ALBERTO ;FIDIA ADVANCED BIOPOLYMERS SRL (IT); PRESSATO D) 4 February 1999 (1999-02-04) page 7, line 25-27; claims 30,32; examples 7-11	1-5, 7-30, 32-36
X	PATENT ABSTRACTS OF JAPAN vol. 017, no. 291 (C-1067), 4 June 1993 (1993-06-04) & JP 05 017369 A (TERUMO CORP), 26 January 1993 (1993-01-26) abstract	1-5,7, 9-12,15, 17-20, 22, 24-30, 32,34-36

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 September 2002	10/10/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Gonzalez Ramon, N

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/19145

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	GOODWIN C A ET AL: "Increased expression of procoagulant activity on the surface of human platelets exposed to heavy-metal compounds." THE BIOCHEMICAL JOURNAL. ENGLAND 15 MAY 1995, vol. 308 (Pt 1), 15 May 1995 (1995-05-15), pages 15-21, XP008007678 ISSN: 0264-6021 figure 3; table 1	1-5,7,9, 11,12
X	KANEKO H ET AL: "Mercury compounds induce a rapid increase in procoagulant activity of monocyte-like U937" BRITISH JOURNAL OF HAEMATOLOGY, OXFORD, GB, vol. 87, no. 1, May 1994 (1994-05), pages 87-93, XP002106328 ISSN: 0007-1048 abstract; figure 2 page 88, column 2, paragraph 2	1-5,7,9, 11,12
X	DE 35 23 023 A (RUHLAND NACHF GMBH DR) 8 January 1987 (1987-01-08) claim 2; examples 1,3	1-9, 12-14, 35,36
X	EP 0 956 869 A (HOGY MEDICAL CO LTD) 17 November 1999 (1999-11-17) page 3, line 30-40 - line 51-55; claim 1; example 2	1,7, 10-12, 15,17, 20, 24-26, 32,35,36
A	EP 0 437 095 A (JOHNSON & JOHNSON MEDICAL) 17 July 1991 (1991-07-17) page 18; claim 3; examples 2,6	1-36
A	US 5 413 786 A (ANRAKU HIDEO) 9 May 1995 (1995-05-09) examples 2,3-1,4-1; table 2	1-36

INTERNATIONAL SEARCH REPORT

ırnational application No.
PCT/US 01/19145

Box I Obs rvati ns where certain claims were found unsearchable (C ntinuati n of item 1 of first sh et)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 15-34, 36 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: partially 1-36
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: partially 1-36

Present claims 1-36 relate to compounds/compositions defined by reference to a desirable characteristics or properties, namely " procoagulant biopolymer", "procoagulant metal ion", "at a level below its effective hemostatic concentration in the absence of..." (claims 1,17,27,35); "insoluble salt comprising said metal ion" (claims 4, 29); " a procoagulant venom", "a plasminogen activator inhibitor","a selectin", " a protease" (claims 7,9,20,22,32,34)
The claims cover all compounds/compositions having these characteristics or properties, whereas the application provides support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT for only a very limited number of such compounds/compositions. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible.
Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compound/compositions by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the compounds/compositions prepared in the examples and those specifically mentioned in claims 9,22,34 with due regard to the general idea underlying the present application.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

formation on patent family members

International Application No
PCT/US 01/19145

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9904828 A	04-02-1999	IT PD970170 A1	28-01-1999
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		EP 0956869 A2	17-11-1999
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		AU 639288 B2	22-07-1993
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		BR 9100114 A	22-10-1991
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